TESTBIOTECH Background 10 - 12 - 2011

Testbiotech comment on EFSA, 2011, Scientific Opinion on application for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto.

Christoph Then & Andreas Bauer-Panskus

Molecular data

There is a complete lack of metabolomic data as well as data showing to which extent the gene activity of plant genes is affected by the artificial introduction of gene constructs.

These data would be highly relevant, since it cannot be denied that there are significant unintended changes in the composition of components (such as Vitamin B1, fatty acids, amino acids, zinc and lignin) and significant unexpected differences in phenotype (such as height, seedling vigour and yield).

Comparative assessment

The comparative assessment is flawed because of biased interpretation of the existing data. There were significant differences in plant components (such as Vitamin B1, fatty acids, amino acids, zinc and lignin) that clearly indicate unintended and unexpected changes in plant metabolism and plant composition in comparison with the isogenic lines. Given these findings, a detailed study of changes in gene activity and plant metabolism should be performed under various and defined environmental stress factors to examine genetic stability of the plants, and to investigate to which extent unintended compounds can emerge in the plant tissue. This is also relevant for the expression data of the newly introduced gene constructs.

The EFSA opinion stating that the changes in plant composition are within the range of historical data is not a sufficient indication for the safety of these crops. Instead, there must be more investigation into *why* there are significant differences in plant composition in comparison with the isogenic lines to avoid major uncertainties (Hilbeck et al 2011). Only after further detailed examination can these data be interpreted regarding potential risks. It also has to be stated that there is no reference to the historical data mentioned by EFSA.

Toxicology

EFSA opinion is based on assumptions about the mode of action of Bt toxins that are not sufficiently based on scientific evidence. There are several modes of action described and not just one theory about how these toxins function. Some of these publications show that selectivity cannot be assumed without detailed testing. Others show that synergistic interactivity has to be taken into account.

In general, the mode of action of Bt toxins is not fully understood. This is even a matter of

controversial debate (Pigott & Ellar, 2007). Strict selectivity of the Bt toxins is not shown by empirical evidence but deduced from its mode of action as described previously. More recent research (Soberon et al., 2009) shows that there are mechanisms that might cause toxicity in other species and even in mammals. As Pardo Lopez et al. (2009) and Pigott et al. (2008) show, synthetically derived and modified Bt toxins can show much higher toxicity than native proteins. Even small changes in the structure of the proteins can cause huge changes in toxicity. Thus, risks for human health cannot be excluded by assumptions or considerations but only by empirical testing before market authorisation.

EFSA did not elaborate on these partially contradictory theories on the mode of action of Bt toxins. No detailed study was performed on the potential impact of Cry3Bb1 on mammalian cells. No assessment of synergies and accumulated effects was presented. The only synergy that is discussed is between the *enzyme* EPSPS that confers resistance to glyphosate and the Cry3Bb1 toxin. But from perspective of toxicology, the potential synergies between the Cry3Bb1 toxin and the formulations (and metabolites) of *glyphosate* used for spraying the plants are much more relevant. There were no tests carried out to examine potential synergies.

Synergistic effects can become highly problematic for non- target organisms. Interaction of the toxins with each other or with other compounds can cause higher toxicity and lower selectivity (Then, 2010). These effects may impact human and animal health as well as the protection of the ecosystems. Some plant enzymes that diminish the digestion of proteins (protease inhibitors) can strongly enhance the toxicity of Bt toxins (Pardo Lopez et al., 2009). Even the presence of very low levels of protease inhibitors can multiply the insecticidal activity of some Cry toxins. It is known that maize produces such inhibitors (Shulmina et al., 1985).

In this case, resistance to glyphosate (brand names such as Roundup) is combined with the insecticide. This leads to a combination of potentially hazardous residues from spraying. In this context, the additive POEA also has to be taken into account because it is even more toxic than glyphosate in the plants (BVL, 2010). The toxicity of glyphosate is currently under revision by the EU. Several experts are warning that toxicity could be higher than expected (Antoniou, et al., 2010; Benachour et al. 2007; Paganelli et al., 2010; PAN AP, 2009). Since the revision of glyphosate under pesticide legislation is not finalized, cultivation of these plants cannot be allowed.

In general, basic prerequisites have to be met to enable proper risk assessment. If these data are not available, hardly any feeding trial or other toxicological test can be designed, performed and interpreted in a meaningful way.

One of these prerequisites is sufficient data on the expression of the newly expressed proteins. But in the case of Bt toxins, standardized protocols to achieve results that can be reproduced by other laboratories are largely missing (Székács et al., 2011). Further, it is not clear how these plants and the expression rate of the newly introduced proteins will be influenced by more extreme weather conditions such as drought or other environmental factors. There are also no data on gene expression in volunteers that can remain after cultivation. Further, the impact from the genetic background of certain varieties has to be taken into account. Several investigations show that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005). This can also impact the Bt content in the plants (Then& Lorch, 2008).

Another basic prerequisite for risk assessment in this context are reliable data on residue loads from spraying with glyphosate formulations. The amount of these residues depends on the specific agronomic management being used in the cultivation of the herbicide resistant plants. The fact is that reliable data covering the actual range of residue load in the plants are not available (Kleter et

al., 2011; Then 2011, EFSA 2011b).

It also has be taken into account, that these plants will be cultivated and fed and might be eaten by mixing them with other genetically engineered plants. Tests have to be performed to find potential combinatorial or accumulated effects.

Residues from spraying and from insecticidal toxins can result in permanent long term exposure of humans and animals and therefore relevant studies to examine chronic effects have to be performed. This has become especially relevant because MON863, which also produces the toxin Cry3Bb1, has since shown several significant effects in animal feeding trials that were classified as signs of toxicity (Seralini et al., 2007). So far, there have been no feeding studies over the whole lifetime of animals and none including following generations.

Allergenicity
There are several proteins in maize that can cause allergic reactions. The newly introduced gene construct might, for example, enhance an immune response to endogenous plant protein(s).Targeted studies on potential impact on the immune system are necessary to exclude risks for animals, farmers and consumers as it is known that some Bt proteins react with the immune system.

Nutritional
The outcome of the study showed significant differences that should have been explored further.

Others
Monitoring plan is not sufficient
The protocols used for conducting the measurements of the Bt toxins have not been fully published or evaluated by independent laboratories. As a result, independent institutions can hardly monitor the actual content of Bt concentration in the plants during cultivation or in food and feed products.

No plan for surveillance as required by European regulation was made available that would allow identification of particular health impacts that might be related to the use of these genetically engineered plants in food and feed.

Monitoring of health and environmental effects has to include the risks associated with the spraying of glyphosate formulations and their residues in the plants.

A case specific monitoring should be requested concerning risks for non- target organisms such as *Coleoptera* species.

The usage of existing networks that are not specifically designed to monitor the impact of genetically engineered plants and the introduction of questionnaires to be filled in by farmers are not sufficient to fulfill requirements of general surveillance under practical conditions as foreseen by EU regulations.

MON88017 cultivation does not accord with the aim of sustainable agriculture
The introduction of these plants is likely to foster the spread of rootworm in maize growing areas. The plants do not produce enough toxin in their roots to kill the pest insects with a >99% likelihood. Instead around 4% of the pest insects can be expected to survive. Further, there will be refugee zones covering around 20% of the maize growing areas where no measures will be taken to diminish the population of rootworms. This is very likely to cause the establishment of rootworm populations especially in those areas where the MON88017 plants are grown. Under these conditions, any strategies to extinguish rootworm by crop rotation and other means are bound to

fail. After some years, the pest insects will have developed resistances (as expected by EFSA), and the rootworm will have been established within regions that could have been protected more efficiently by other strategies. In conclusion, the overall strategy behind the introduction of MON88017 does not support sustainable agriculture in the long run.

The same argument is relevant for the impact of large scale application of glyphosate in maize growing regions. Cultivation of these herbicide resistant plants poses risks to biodiversity, plant health, soil fertility and enables the emergence of herbicide resistant weeds (Benbrook, 2009). The massive usage of glyphosate in herbicide resistant crops endangers the health of rural communities, aquatic systems as well as impacting biodiversity and soil fertility. It can cause plant diseases e.g increased infestation with fungal diseases (Johal & Huber, 2009). The negative impact on plant growth and plant health can even be transmitted to other plants cultivated in the same field in the following year (Bott et al., 2011, Bott et al., 2007).

Environmental risk assessment

The EFSA has made assumptions about the mode of action of Bt toxins that are not sufficiently based on scientific evidence. There are several modes of action that are described and not just one theory on how these toxins work. Some of these publications show that selectivity cannot be assumed without detailed testing. Others show that synergistic interactivity has to be taken into account.

In general, it is not fully understood how Bt toxins work. It is a matter of controversial debate (Pigott & Ellar, 2007). Strict selectivity of the Bt toxins is not shown by empirical evidence but deduced from its mode of action as described previously. More recent research (Soberon et al., 2009) shows that there are mechanisms that might cause toxicity in other species and even in mammals.

The EFSA did not elaborate on these partially contradictory theories of mode of action of Bt toxins. No systematic overview was performed concerning the potential impact of these toxins on various non- target organisms. Despite the fact that several studies on non- target organisms have been published more systematic screening of relevant organisms, including wild life species, is necessary to design, perform and evaluate studies on potential impacts on specific non- target organisms. It also should not be left to the applicant to choose the most relevant organisms related to the ecosystems in various geo-climatic regions.

No assessment of synergies and accumulated effects was presented. The only synergy that is discussed is the one between the *enzyme* EPSPS that confers resistance to glyphosate and the Cry3Bb1 toxin. Much more relevant from perspective of toxicology are the potential synergies between the Cry3Bb1 toxin and the *formulations (and metabolites) of glyphosate* used for spraying the plants. Since this is not part of the assessment under pesticide regulation, it has to be assessed during risks assessment of the genetically engineered trait.

Synergistic effects can become highly problematic for non- target organisms. Interaction of the toxins with each other or with other compounds can cause higher toxicity and lower selectivity (Then, 2010). These effects may impact the ecosystems on various levels. For example, it has been shown that slugs incorporate the Cry3Bb1 toxins. It is also known that co-stressors such as cadmium and nematodes can cause toxicity of Cry toxins in slugs (Kramarz etl al., 2007, Kramarz et al., 2009). Nevertheless, this issue was not included in risk assessment. In general, a systematic screening of synergistic or accumulated effects on a sufficiently broad range of organisms has to be performed. This should also include the cultivation of other genetically engineered crops.

In general, to run proper assessment on toxicology, basic prerequisites have to be met. If these data are not available, hardly any assessment of environmental risks can be designed, performed and interpreted in a meaningful way.

One of these prerequisites is sufficient data on the expression of the newly expressed proteins. But in the case of Bt toxins, standardised protocols to measure the content of Bt toxins in a way that the results can be reproduced by other laboratories are largely missing (Székács et al., 2011). Further, it is not clear how these plants and the expression rate of the newly introduced proteins will be influenced by more extreme weather conditions such as drought. There are also no data on gene expression in volunteers that can remain after cultivation. Further, the impact from the genetic background of certain varieties has to be taken into account. Several investigations show that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005). This can also impact the Bt content in the plants (Then& Lorch, 2008).

Since the cultivation of these plants will lead to a long term and large scale exposure of various organisms, adequate studies to examine long chronic effects have to be performed. But in the case of MON88107 most studies were only performed for one year.

Further, most studies were not performed on MON88107 but on other genetically engineered plants that also produce Cry3Bb1. EFSA considered these tests as being comparable because of nearly identical structures of the insecticidal proteins. However, as Saeglitz et al (2006) show, Bt toxins with identical structure but derived from differing sources can vary extensively in their toxicity. Therefore, major uncertainties remain about whether data derived from traits such as MON863 can really be used in the risk assessment of MON88017.

Large-scale cultivation will bring many wildlife species into contact with these plants. Detailed empirical investigations of the organisms in the receiving environments must be conducted and include several tiers of the food web. Bt toxin can accumulate in the food web, reaching higher content than in the genetically engineered plants. But even the risks for most relevant non- target organisms (Coleoptera) were mostly assessed by modeling and not by empirical investigations. The tiered approach as it is applied in risk assessment is too narrow to really exclude risks for ecosystems. For example, risks for wildlife species were not included in risk assessment. The impact on rodents, birds and other animal species should be assessed carefully.

Conclusion and recommendations

By pointing out these major data gaps, the risk assessment as performed by EFSA has to be rejected.

References:

Antoniou, M., Brack, P., Carrasco, A., Fagan, J., Habib, M., Kageyama, P., Leifert, C., Nodari, R. O., Pengue W. (2010) GM Soy: Sustainable? Responsible?, GLS Bank & ARGE gentechnikfrei, http://www.gmwatch.eu/?option=com_content&view=article&id=12479

Benachour, N., Siphatur, H., Moslemi, S., Gasnier, C., Travert, C., Seralini, G. E. (2007) Time- and dose-dependent effects of Roundup on human embryonic and placental cells, Arch Environ Contam Toxicol 53:126-33.

Benbrook, C., (2009) Impacts of Genetically Engineered Crops on Pesticide Use: The First Thirteen Years, www.organic-center.org/reportfiles/13Years20091116.pdf

Bott S., Tesfamariam T., Candan H., Ismail Cakmak I., Römheld V., Neumann G. (2008) Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.), Plant Soil (2008) 312:185–194

Bott, S., Tesfamariam, T., Kania, A., Eman, B., Aslan, N., Roemheld, V. Neumann, G. (2011) Phytotoxicity of glyphosate soil residues re-mobilise4d by phosphate fertilization. Plant Soil 315:2-11. DOI 10, 1007/s11104-010-06989-3.

BVL (2010)
http://www.bvl.bund.de/DE/04_Pflanzenschutzmittel/05_Fachmeldungen/2010/psm_anwendungsbestimmungen_tallowamin-Mittel.html

EFSA (2011a) Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2011;9(11):2428. [152 pp.]
doi:10.2903/j.efsa.2011.2428. Available online: www.efsa.europa.eu/efsajournal

EFSA (2011b): 2009 EU Report on Pesticide Residues. EFSA Journal 2011; 9(11):2430. [226 pp.]
doi:10.2903/j.efsa.2011.2430. Available online: www.efsa.europa.eu/efsajournal

Hilbeck A.,Meier M.,Römbke J.,Jänsch S.,Teichmann H.,Tappeser B., (2011) Environmental risk assessment of genetically modified plants-concepts and controversies.EnvironmentalSciences Europe.2011;23(13).

Johal, G.R. and Huber, D.M. (2009) Glyphosate effects on diseases of plants. European J. Agron. 31:144-152.

Kleter, G.A., Unsworth J.B., Harris C.A. (2011) The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues, wileyonlinelibrary.com, DOI 10.1002/ps.2128

Kramarz, P.E., de Vaufleurey, A., Zygmunt, P.M.S., Verdun, C., (2007) Increased response to cadmium and Bt maize toxicity in the snail Helix aspersa infected by the nematode Phasmarhabditis hermaphrodita. Environmental Toxicology and Chemistry 26: 73–79

Kramarz, P., de Vaufleury, A., Gimbert, F., Cortet, J., Tabone, E., Andersen, M., Krogh, P. (2009) Effects of Bt-Maize Material on the Life Cycle of the Land Snail Cantareus aspersus. Applied Soil Ecology 42: 236–242

Matthews D., Jones H., Gans P., Coates St., Smith L.M.J. (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. Journal of Agricultural and Food Chemistry, 10.1021/jf050589r.

Paganelli, A., Gnazzo, V., Acosta, H., López, S. L., Carrasco, A. E. (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. Chem. Res. Toxicol., August 9. pubs.acs.org/doi/abs/10.1021/tx1001749

PAN AP, Pesticide Action Network Asian Pacific (2009) Monograph on Glyphosate, www.panap.net/en/p/post/pesticides-info-database/115

Pardo-López, L., Muñoz-Garay,C., Porta, H., Rodríguez-Almazán,C., Soberón M., Bravo A. (2009) Strategies to improve the insecticidal activity of Cry toxins from Bacillus thuringiensis, Peptides, 30(3): 589–595. doi:10.1016/j.peptides.2008.07.027.

Pigott, C.R. & Ellar, D.J. (2007) Role of Receptors in Bacillus thuringiensis Crystal Toxin Activity: Microbiol Mol Biol Rev 71 (2): 255–281

Pigott, C.R., King, S.M., Ellar D.J. (2008) Investigating the Properties of Bacillus thuringiensis Cry Proteins with Novel Loop Replacements Created Using Combinatorial Molecular Biology, Applied and Enviromental Microbiology: 3497–3511

Saeglitz, C., Bartsch D., Eber, A., Gathmann, K., Priesnitz, U., Schuphan, I. (2006) Monitoring the Cry1Ab Susceptibility of European Corn Borer in Germany, J. Econ. Entomol. 99(5): 1768Ð1773

Séralini G.-E., Cellier D. & Spiroux de Vendomois J. (2007) New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Archives of Environmental Contamination and Toxicology 52, 596-602.

Shulmina AI, Dronova LA, Shubin VV, et al. (1985) Determination of the chymotrypsin inhibitors, secondary structure of the chymotrypsin inhibitor from corn by the circular-dichroism method. BIOCHEMISTRY-MOSCOW 50, 7: 980-982

Soberón, A., Gill, S.S., Bravo A. (2009) Signaling versus punching hole: How do Bacillus thuringiensis toxins kill insect midgut cells? Cell. Mol. Life Sci. 66 (2009) 1337 – 1349

Székács, A., Weiss, G., Quist, D., Takács, E., Darvas, B., Meier, M., Swain T., Hilbeck A., (2011) Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay, Food and Agricultural Immunology, DOI:10.1080/09540105.2011.604773.

Then, C. (2010) Risk assessment of toxins derived from Bacillus thuringiensis-synergism, efficacy, and selectivity. Environ Sci Pollut Res Int; 17(3):791-7

Then, C. (2011) Vorsicht „Giftmischer“: Gentechnisch veränderte Pflanzen in Futter-und Lebensmitteln, ein Testbiotech-Report, http://www.testbiotech.de/sites/default/files/Testbiotech_Giftmischer_April_2011.pdf

Then C. & Lorch A. (2008) A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce?: in Breckling B, Reuter H, Verhoeven R (eds) (2008) Implications of GM-Crop Cultivation at Large Spatial Scales., Theorie in der Ökologie 14. Frankfurt, Peter Lang, http://www.gmls.eu/index.php?contact=ja